

Supplemental Figures

The following data are provided as supplementary figures:

- SDS_PAGE of a representative panel of full length human proteins cloned and expressed in *E. coli* and used for microarray printing (Supplementary Fig. 1);
- Histogram of the control viral and bacterial proteins printed on the arrays (Supplementary Fig. 2);
- Plot of the IgG curve used for array normalization and distance matrix of the slides used for array quality check (Supplementary Fig. 3A and B);
- Boxplots of signal intensities with the validation sample set of the 16 antigens selected by the comparison of AIH with HCV patients in the first phase of the study (Supplementary Fig. 4);
- Predictive analysis of Microarray (PAM) (Supplementary Fig. 5);
Correlation between fluorescence signal intensities of patients sera reacting with IL4R domain in DELFIA assay and the percentage of Stat6 phosphorylation observed in cells pre-incubated with the same sera and stimulated with IL4 (Supplementary Fig. 6);
- Titration of Stat6 phosphorylation inhibition by patients sera (Supplementary Fig. 7);
- Relative quantification of soluble IL4 Receptor in sera of patients and healthy donors (Supplementary Fig. 8).

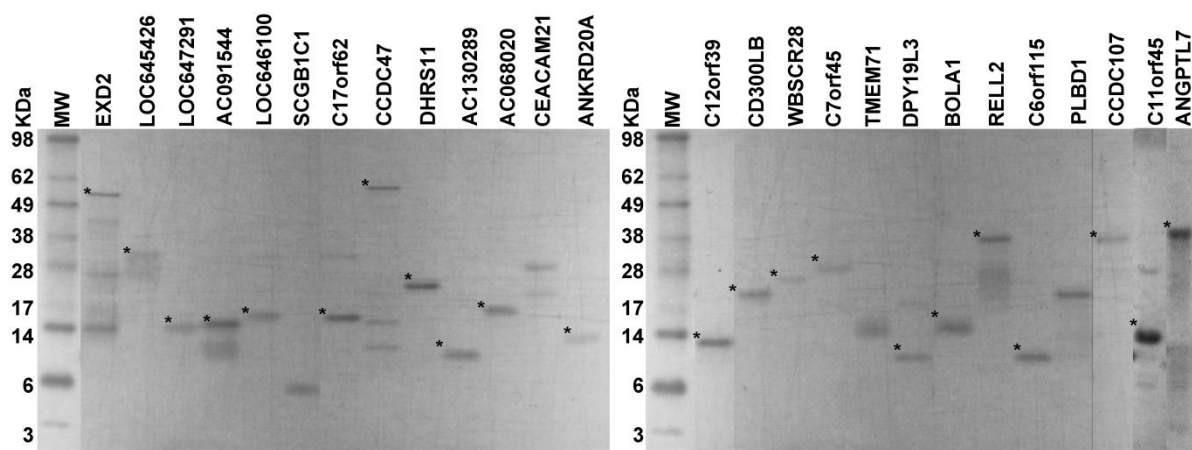


Figure S1.

Coomassie blue stained SDS-PAGE gels of recombinant human proteins. Representative panel of 26 full length recombinant human proteins, expressed in E.coli, and run on SDS-PAGE gels to assess their purity and integrity. Almost all proteins showed a band of the expected molecular weight (Asterisks mark).

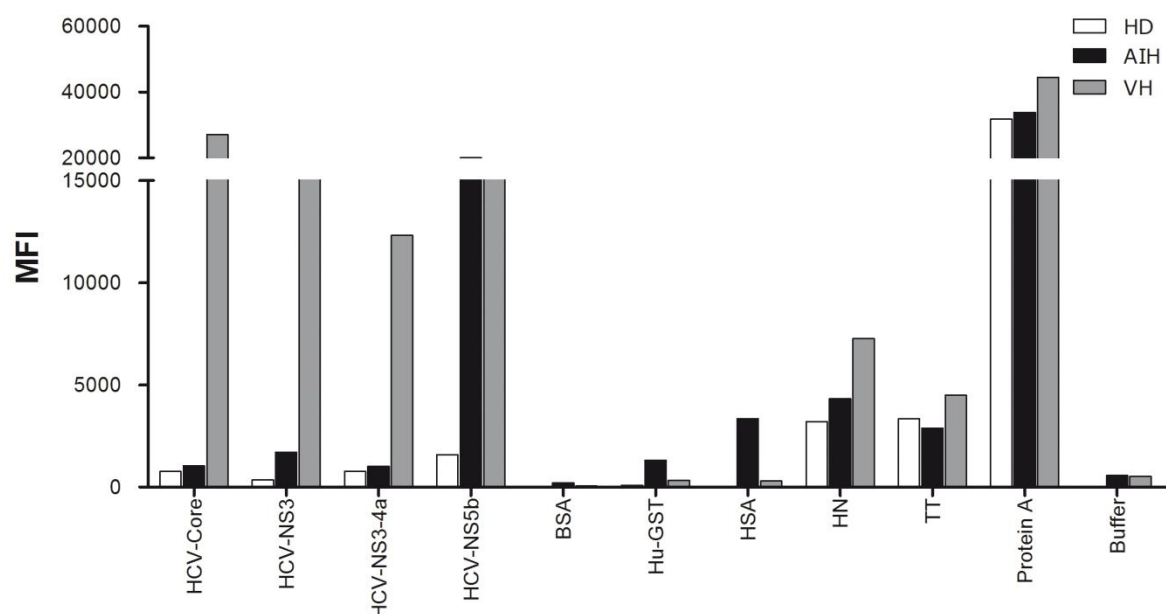
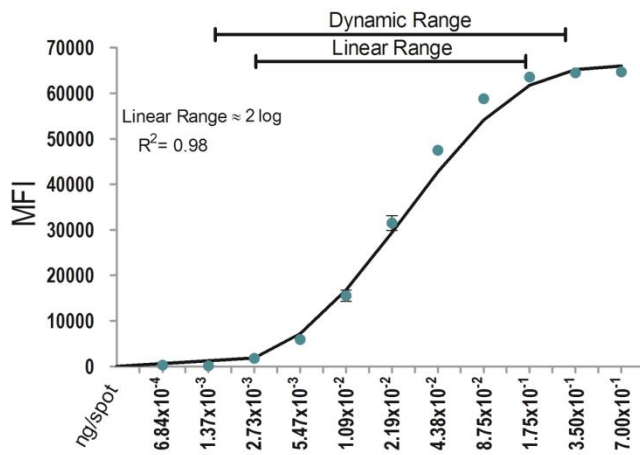
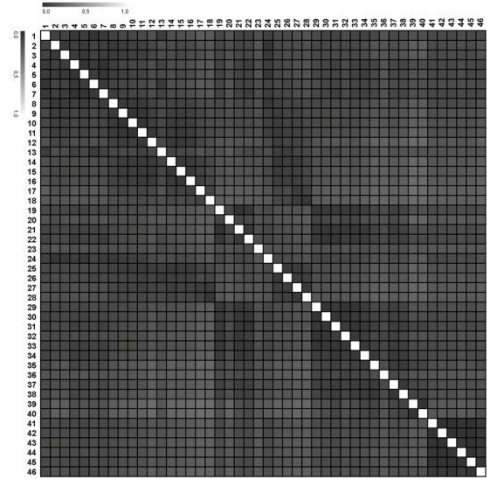


Figure S2. Bar-chart of control viral and bacterial proteins printed on the arrays. Protein array MFI values of control proteins reacting with sera from patients with Viral Hepatitis (VH), Autoimmune Hepatitis (AIH) and healthy donors (HD). In particular, MFI values of the following proteins are reported: 4 HCV proteins (HCV Core protein and Non-structural proteins NS3, NS3-4a, NS5b), Tetanus toxin (TT), H1N1 influenza antigen, Bovine Serum Albumin (BSA), Human Serum Albumin (HSA), Human Glutathione-S-Transferase (Hu-GST) and Protein A from *S. aureus*.

a**b****Figure S3.**

Microarray normalization and quality check. (A) Plot of human IgG curve used for protein array data normalization. The dots correspond to the average MFI signal detected for each IgG concentration, while the solid line represents the interpolated resulting sigmoid curve. (B) Distance matrix of 46 protein microarrays hybridized with anti-His mAb. The distance between two slides, inverse expression of similarity, is calculated using Pearson's metric. Average Distance = 0.27 ± 0.11 .

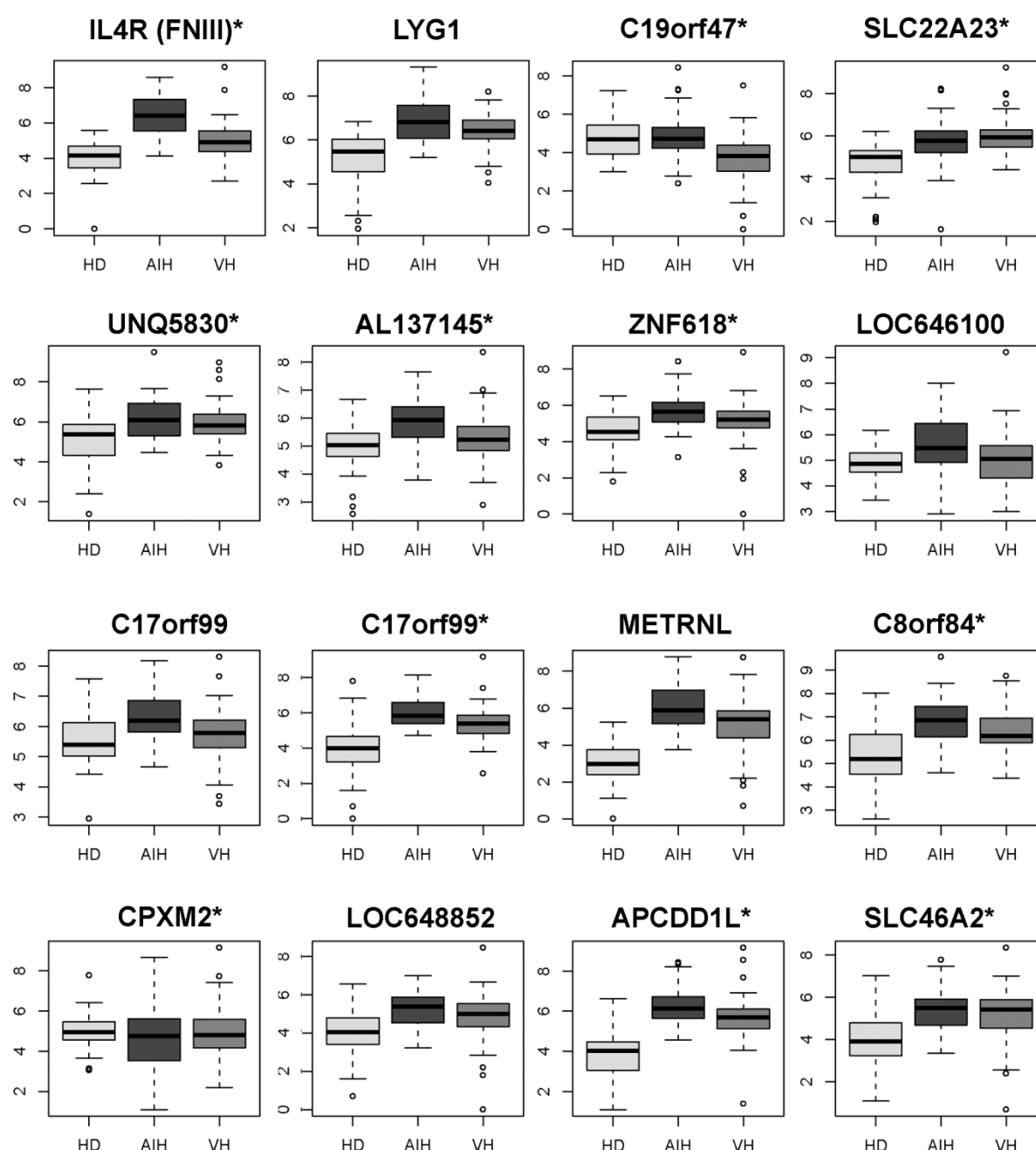
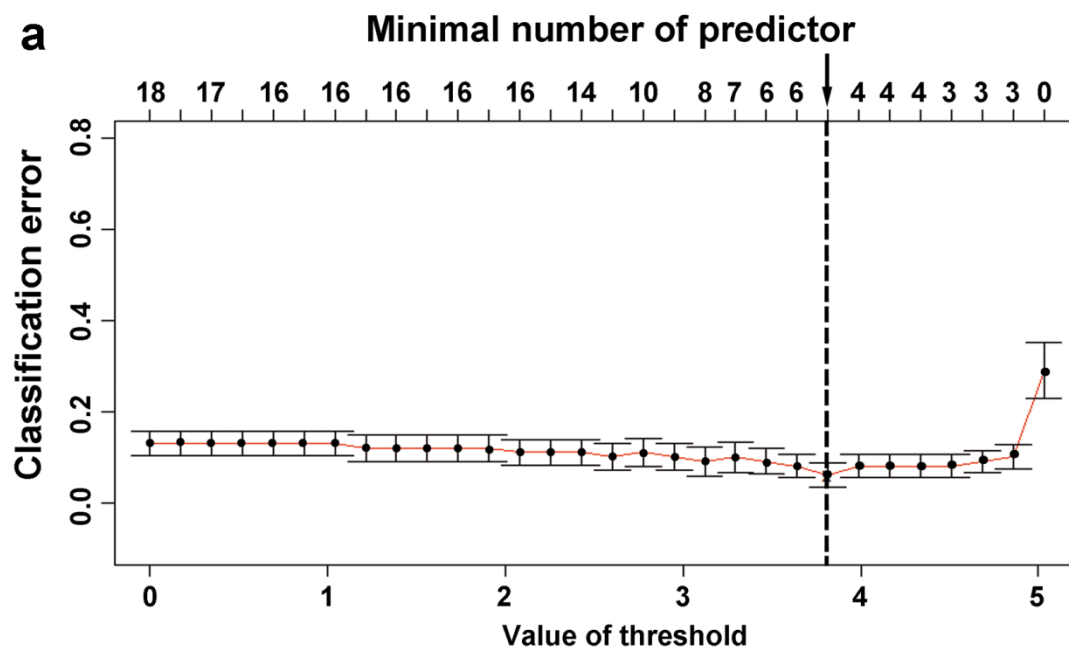


Figure S4.

DELFI[®] assay for the 16 AIH autoantigen candidates selected in the Discovery phase. Boxplots of log-transformed MFI values of the 16 antigens tested with Validation sera from 50 AIH patients, 50 healthy donors (HD), and 74 patients with either B or C chronic viral hepatitis (VH). The boxes define the interquartile range (IQR). The extreme outliers beyond the median+ 1.5 IQR are showed as dots. Asterisk: proteins cloned and expressed as domains.



b

ID	HD-score	AIH-score	Average rank in CV
APCDD1L*	-0.1214	0.1214	1.5
METRNL	-0.1147	0.1147	2.1
IL4R (FNIII)*	-0.1124	0.1124	2.4
C17orf99*	-0.0532	0.0532	4

Figure S5.

Predictive analysis of microarray (PAM). (A) Semi-supervised prediction analysis performed with PAM algorithm on the 16 autoantigen candidates. After internal 10-fold cross validation, a threshold of 3.816 was selected as the one giving the lowest misclassification error. (B) The AIH-score, HD-score and Average rank in cross validation are reported for the 4 antigens selected as best classifiers by the above threshold. Asterisk: proteins cloned and expressed as domains.

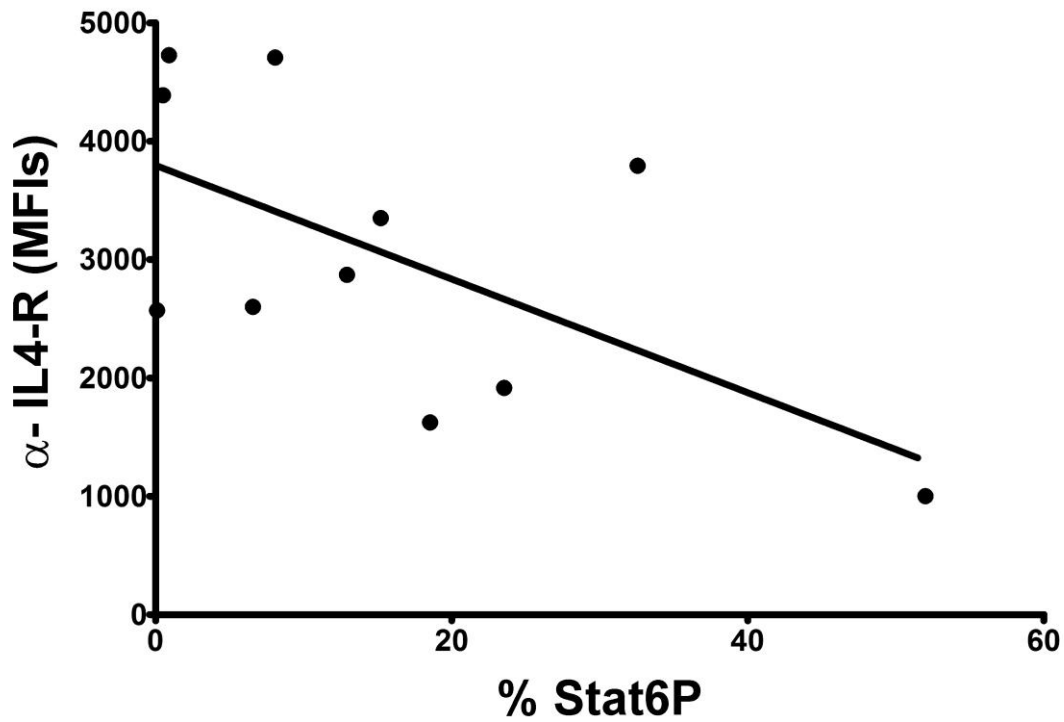


Figure S6.

Correlation between the signal intensity of anti-IL4R reactivity of AIH patients sera and their ability to inhibit Stat6 Phosphorylation. The MFI signals of patients sera to IL4R domain measured by DELFIA® are plotted against the percentage of Stat6P positive CD4+ T cells pre-incubated with patients sera and subsequently stimulated with IL4. The correlation was statistically significant (p val<0.05).

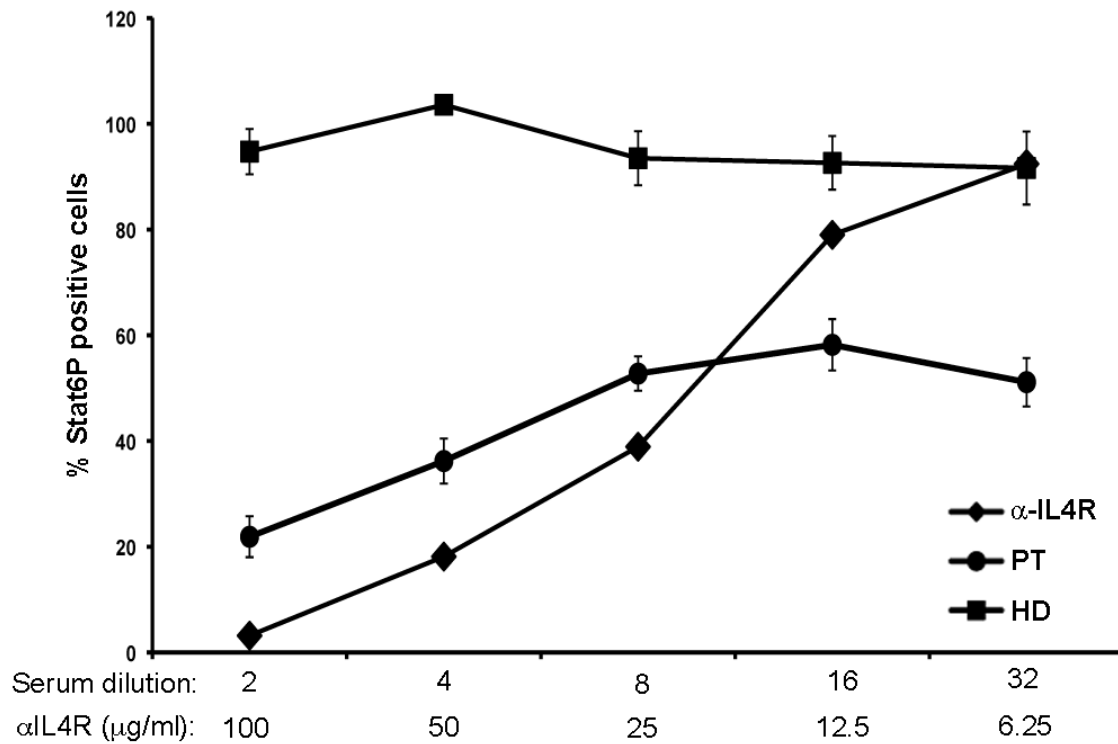


Figure S7.

Titration of Stat6 Phosphorylation inhibition by AIH patients sera. Sera from 10 AIH Patients and 4 HD were titrated for their ability to inhibit Stat6 Phosphorylation induced by IL4 on CD4+ T cells. Results are expressed as percentage of Stat6 P positive cells after pre-incubation with (◆) a neutralizing anti-IL4R polyclonal antibody, (●) AIH patients sera, (■) HD sera, and subsequent stimulation with IL4. Error bars: standard error.

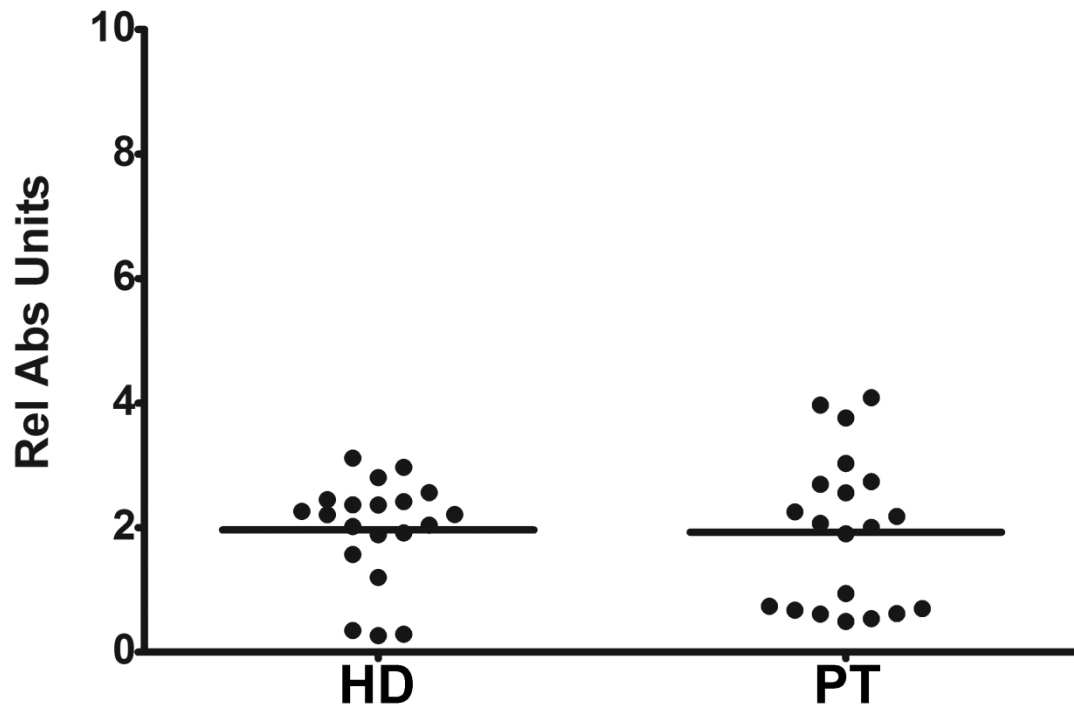


Figure S8.

AIH and HD display similar levels of serum soluble IL4R. The amount of sIL4R in sera from 20 AIH patients and 20 HD was measured by ELISA. Results are expressed as relative absorbance values. Difference between AIH and HD is not statistically significant.